**Brief Definitive Report**

# CYCLIC ADENOSINE MONOPHOSPHATE RESPONSE TO PROSTAGLANDIN $E_2$ ON SUBPOPULATIONS OF HUMAN LYMPHOCYTES*

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Small molecular weight hormones such as prostaglandin E (PGE) and histamine, would appear to have a role in the regulation of cellular immune function. Evidence for this is as follows. $PGE_1$ or $E_2$ added in low concentrations ($10^{-9}$–$10^{-6}$ M) to mitogen-stimulated cultures of human peripheral blood mononuclear cells (PBMC) or T cells causes inhibition of proliferation as measured by [$^3$H]thymidine incorporation (1, 2) or by cell numbers (3). $PGE_2$ is produced in mitogen-stimulated cultures of human PBMC (1, 2) and mouse splenocytes (4). Blockade of PG synthesis in mitogen-stimulated cultures by addition of various PG synthetase inhibitors results in an increase in [$^3$H]thymidine incorporation and in cell numbers (3). Administration of PG synthetase inhibitors in vivo also results in an enhanced delayed hypersensitivity skin test reponse in man (5) and in experimental animals (6).

A role for histamine in immunoregulation is suggested by the appearance of histamine receptors on activated T lymphocytes (7). These T cells, which bear receptors for histamine, act as suppressor cells in several systems (8–10).

We have recently demonstrated high-affinity binding sites for $PGE_2$ on human lymphocytes (11, 12). We have also confirmed earlier reports (13) that cyclic AMP is the second messenger for $PGE_2$ in lymphocytes (12). The cyclic AMP response to $PGE_2$ and histamine provides a sensitive assay which indicates the presence of receptors for these compounds (12, 13).

In this report we show that the cyclic AMP response to $PGE_2$ is not equally distributed among lymphocytes. It would appear that both B and T cells respond to $PGE_2$, but among T cells, only those that bear a receptor for the Fc portion of IgG ($T\gamma$ cells) respond to $PGE_2$ with a cyclic AMP increase.

## Materials and Methods

*Isolation of Mononuclear Cells.* Heparinized peripheral venous blood was obtained from healthy donors. Mononuclear cells were isolated on a Ficoll-Hypaque density gradient (14) (Pharmacia Fine Chemicals, Piscataway, N. J.). Glass adherent cells were removed by incuba-

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tion at 37°C for 30 min in glass Petri dishes. Glass nonadherent cells were washed three times with Hanks' balanced salt solution (HBSS) and resuspended at $4 \times 10^6$ cells per milliliter in RPMI-1640 medium plus 20% fetal calf serum (absorbed with sheep erythrocytes [sRBC]).

*Isolation and Purification of T Lymphocytes.* Equal volumes of glass nonadherent cells were mixed with 1% neuraminidase-treated sRBC (10 IU neuraminidase per 1 ml of 5% sRBC). The mixture was incubated for 10 min at 37°C, centrifuged for 5 min at 200 *g*, and then incubated for 1 h at 4°C. After incubation, the pellets were resuspended gently using a wide bore pipette. Rosetting T cells were purified from nonrosetting cells on a Ficoll-Hypaque gradient by centrifugation for 20 min at 480 *g* in room temperature. Sheep RBC were lysed with Tris-$NH_4Cl$ buffer. Cells from the bottom were washed three times in HBSS and were shown to contain 96–98% T cells as determined by rerosetting with sRBC and lack of surface immunoglobulins as identified by immunofluorescence using rabbit $F(ab')_2$ anti-human Ig. Viability was >98% as judged by trypan blue dye exclusion. Cells at the interface, termed non-T cells, were composed of 50–60% cells bearing surface immunoglobulin and 15–25% E-rosetting cells.

*Isolation of Tγ Lymphocytes.* Tγ cells were isolated on the same day by using bovine erythrocytes coated with the IgG fraction of rabbit anti-bovine RBC antibody (N. L. Cappel Laboratories, Inc., Cochranville, Pa.). Bovine RBC-antibody complexes (EA-IgG) were prepared according to Gupta and Good (15) by incubating equal volumes of 2% bovine RBC and IgG antibody (1:200) at room temperature for 90 min. The complexes were washed three times with HBSS and resuspended to 1% in RPMI-1640 medium. Purified T cells ($4 \times 10^6$ per milliliter in RPMI-1640) were mixed with equal volumes of 1% EA-IgG, centrifuged at 200 *g* for 5 min, and incubated for 60 min at 4°C. The pellets were resuspended using Pasteur pipettes, layered over a Ficoll-Hypaque gradient and centrifuged at 400 *g* for 20 min in room temperature. Greater than 96% of the cells at the bottom rosetted with EA-IgG; 2–4% of the cells at the interface rosetted with EA-IgG. Bovine RBC were lysed using Tris-$NH_4Cl$ buffer.

*Drugs.* $PGE_2$ was a gift of Dr. John Pike (The Upjohn Co., Kalamazoo, Mich.) and was dissolved in 95% ethyl alcohol with a final ethanol concentration in the incubation medium of ≤0.001%. 0.01% ethanol had no effect on the cyclic AMP response of control incubations. Histamine (Sigma Chemical Co., St. Louis, Mo.) was dissolved in HBSS.

*Generation of Cyclic AMP.* Each lymphocyte fraction was resuspended at a final concentration of $10^6$ per milliliter in minimal essential media (Microbiological Associates, Walkersville, Md.) with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.) supplemented with L-glutamine and penicillin-streptomycin. 1-ml aliquots of the cell suspensions were incubated in polypropylene tubes (2063, Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Ca.) at 37°C with 5% $CO_2$ for 60 min. Drugs were then added and reactions were stopped after 5 min additional incubation. Preliminary experiments showed that a 5-min incubation period gave maximal cyclic AMP responses for both prostaglandin $E_2$ and histamine when no phosphodiesterase inhibitor was present in the incubation mixture.

Incubations were stopped by centrifugation at 4°C at 400 *g* for 5 min. After centrifugation, the supernates were decanted and the tubes were blotted against absorbent material. The cells were resuspended in 1 ml of cold Na acetate, 50 mM, pH 6.2, vortexed, and frozen in liquid nitrogen for 5 min. The tubes were then placed in a boiling water bath for 5 min. The resultant cell debris was removed by centrifugation at 2,000 *g* for 20 min, and the supernates were assayed for cyclic AMP content using an acetylation method as described by Frandsen and Krishna (16). All lymphocyte incubations were prepared in duplicate. Duplicates of each were assayed for cyclic AMP.

## Results and Discussion

Table I presents data from three normal subjects on the cyclic AMP response of lymphocytes to $PGE_2$ and histamine. Unfractionated lymphocytes, T-cell-enriched, T-cell-depleted, Tγ, and non-Tγ fractions all show a substantial cyclic AMP increase after exposure to PGE at $10^{-6}$ M. The cyclic AMP response of the T cells to $PGE_2$ would appear to be concentrated in the Tγ fraction. The T-cell fraction, of which 11–18% were Tγ cells, had a 10.8-fold increase in cyclic AMP after exposure to $PGE_2$. The Tγ fraction had a 27.4-fold increase in cyclic AMP with exposure to $PGE_2$,

TABLE I

*Cyclic AMP Response to $PGE_2$ and Histamine in Human Lymphocytes and Lymphocyte Subfractions*

| Cells tested | Base-line cyclic AMP | $PGE_2$ $10^{-6}$ M | | Histamine $10^{-4}$ M | |
|---|---|---|---|---|---|
| | | Cyclic AMP | SI* | Cyclic AMP | SI* |
| | *fmol/$10^6$ cells* | *fmol/$10^6$ cells* | | *fmol/$10^6$ cells* | |
| Lymphocytes | 2,485 ± 760 | 31,160 ± 6,830 | 12.8 ± 3.8 | 3,510 ± 1,095 | 1.4 ± 0.2 |
| T-depleted | 4,955 ± 1,440 | 51,250 ± 10,910 | 11.8 ± 1.5 | 9,255 ± 2,740 | 1.8 ± 0.2 |
| T-enriched | 2,620 ± 440 | 29,120 ± 8,035 | 10.8 ± 2.2 | 3,405 ± 900 | 1.2 ± 0.3 |
| Tγ | 2,460 ± 860 | 64,975 ± 21,335 | 27.4 ± 3.2 | 2,110 ± 400 | 1.0 ± 0.2 |
| Non-Tγ | 1,065 ± 240 | 4,365 ± 1,510 | 4.0 ± 1.6 | 720 ± 140 | 0.7 ± 0.4 |

Data are from three experiments on different individuals. The cyclic AMP values are the mean ± standard error in fmol/$10^6$ cells. Each incubation was done in duplicate, and the cyclic AMP determination was also in duplicate.

* SI, stimulation index. Data for SI are given as mean ± standard error of the individual stimulation indices. The stimulation indices for the lymphocyte, T-depleted, and T-enriched fractions after $PGE_2$ are not different from one another ($P > 0.5$). The SI for Tγ is significantly greater ($P < 0.001$), and the SI for the non-Tγ is significantly smaller ($P < 0.01$) than the SIs for the lymphocyte, T-depleted, or T-enriched fractions after $PGE_2$.

whereas the T cells remaining after enrichment for Tγ had only a fourfold increase in cyclic AMP. The mean absolute increase in cyclic AMP content after $PGE_2$ stimulation was 19 times higher in the Tγ than in the non-Tγ fraction. Because the non-Tγ fraction still had 2–4% Tγ cells, the small cyclic AMP increase seen in the non-Tγ fraction could be almost entirely explained by the contaminating Tγ cells.

The cyclic AMP response of the lymphocyte fractions to histamine was more modest. A small but significant rise in cyclic AMP occurred in the response of unfractionated lymphocytes to histamine. The non-T-cell fraction also had a small response, whereas the T-enriched, Tγ, and non-Tγ fractions showed no cyclic AMP response to histamine. These studies were performed without a phosphodiesterase inhibitor in the cell suspensions. When $10^{-4}$ M 3-isobutyl-1-methylxanthine, a powerful phosphodiesterase inhibitor, was added to the suspensions before the addition of histamine, there was a small but significant increase in cyclic AMP in the T-cell fraction (stimulation index of 4.4 ± 1.2) with similar increases in both the Tγ and non-Tγ fractions (3.1 ± 0.6 and 3.6 ± 0.8, respectively). Our finding of a lack of histamine receptors on unstimulated lymphocytes is in agreement with the findings of previous investigations (1, 5). Interestingly, the weak cyclic AMP response to histamine in the presence of a phosphodiesterase inhibitor was not concentrated in any lymphocyte subfraction.

It is possible that the binding or activation of the Fc receptors of the T cells during the isolation procedure rendered the cells more sensitive to $PGE_2$. To test for this we added EA-IgG to T-cell suspensions and measured the effect on the cyclic AMP response of these T cells to $PGE_2$. In three experiments, the stimulation index for cyclic AMP after exposure to $10^{-6}$ M $PGE_2$ was 9.4 ± 2.1 for the T cells; this increased to 14.8 ± 1.8 for the T cells plus bovine EA-IgG. Thus, not only are Tγ cells responsible for the cyclic AMP increase to $PGE_2$ in T cells, but the Tγ cells are further sensitized to $PGE_2$ by activation of their Fc receptors. It was not possible to measure the cyclic AMP response of Tγ cells to PGE without activating the Fc receptors. The negative isolation technique of enriching for Tγ cells by rosetting Tμ cells (those T cells with an Fc receptor for IgM) involves an incubation step of 18 h. We have previously shown that lymphocytes lose their response to PGE during such an incubation (11, 12).

Lymphocytes that are incubated at 37°C for 20 h lose their sensitivity to $PGE_2$, as measured by suppression of mitogenesis (2), [$^3$H]PGE binding (11), or cyclic AMP response (12). Because the Tγ cells are the T cells that respond to PGE with a cyclic AMP increase, we examined whether the loss of cyclic AMP response to $PGE_2$ with preincubation was a result of a loss of Tγ cells. In three experiments, incubation of Tγ cells for 20 h at 37°C before exposure to $PGE_2$ resulted in a loss of cyclic AMP response to $10^{-6}$ M $PGE_2$ (stimulation index of 27.4 ± 3.2 before incubation vs. 3.4 ± 0.8 after incubation). This loss of cyclic AMP response was paralleled by the disappearance of the Tγ cells from the cultures. Before incubation, >90% of the cells in the Tγ fraction formed rosettes with EA-IgG. After 20 h of incubation, this percentage decreased to 20 ± 1%. Thus, the loss of sensitivity of lymphocytes to PGE with preincubation may be a result of a loss of Tγ cells.

The localization of the cyclic AMP response to PGE on only a small fraction of peripheral blood T cells suggests new possibilities for how PGE suppresses the T-cell response to mitogens. We have previously shown that PGE will inhibit [$^3$H]thymidine incorporation in phytohemagglutinin- or concanavalin A-stimulated T cells by as much as 90% (1). Because >50% of circulating T cells proliferate in response to these mitogens (17), it is difficult to postulate that PGE is directly suppressing the proliferative response of each T cell in light of our finding that only a small proportion of these cells appear to bear PGE receptors. It is possible that PGE activates the Tγ population to suppress the proliferative response of the non-Tγ cells. Such a mechanism of PGE activation of suppressor cell function has previously been suggested by Webb and Nowowiejski in mouse splenocytes (4).

## Summary

Receptors for prostaglandin $E_2$ or histamine were measured on subpopulations of human lymphocytes, using the cyclic AMP increase after exposure to prostaglandin or histamine as an indicator for the presence of receptors. The cyclic AMP response to prostaglandin $E_2$ was similar in unfractionated lymphocytes and the T-enriched and T-depleted fractions. Within the T-enriched population, T cells bearing a receptor for the Fc portion of IgG (Tγ cells) had a 27.4-fold rise in cyclic AMP after exposure to prostaglandin $E_2$, whereas the remaining T cells (non-Tγ cells) had a fourfold increase. It would appear that prostaglandin receptors are concentrated on a small subfraction of Tγ cells, comprising ~15% of the T-cell population. The cyclic AMP response to histamine was <twofold in all lymphocyte fractions.

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